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(54) Title: A METHOD OF TESTING A CELL SAMPLE

(57) Abstract

The present invention measures agglutination using a process which is capable of testing how tightly agglutinated cells are bonded by measuring how much force is required to separate them. By causing red cells which are approximately biconcave discs to sphere, the effective surface area available for bonding diminishes. Sphering a cell increases the space between antigen binding sites and increases the mean distance across which bonding occurs. As the surface area available for bonding between cells decreases as cells sphere they lose bonding strength, thus allowing clumped cells to separate. By recording the inducing pressure and the number of cells (or quantities related to it) as they change with respect to the inducing pressure, agglutination can be detected, quantified and monitored. This provides a simple but effective test for blood grouping and cross–matching by introducing an appropriate source of antibodies and detecting whether or not agglutination occurs.

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A METHOD OF TESTING A CELL SAMPLE

Background to the Invention

All types of blood cells occasionally agglutinate spontaneously, frequently heralding a serious haemolytic disease. It may indicate an underlying malignancy such as non-Hodgkin's lymphoma, Hodgkin's disease, acute lymphocytic leukaemia, carcinoma, thymoma and ovarian tumours. It occurs in blood group incompatibility as in haemolytic disease of the newborn, and mis-matched blood transfusions; also in paraoxysmal nocturnal haemoglobinuria and hypogammaglobulinemia; in some collagen diseases such as disseminated lupus erythematosis, rheumatoid arthritis, ulcerative colitis and hepatitis; in some infections such as viral and Mycoplasma pneumonia, cytomegalovirus, tuberculosis and infectious mononucleosis, and as a toxic reaction to some drugs such as L-dopa. As the presence of intra or extra vascular haemolysis in these diseases carries at least a 10% mortality, the identification of red cell agglutination is useful for the early diagnosis and for monitoring the response to treatment.

Traditionally, agglutination is detected by visually observing clumped cells. Whilst automated cell counters have supplanted all manual routine haematology they cannot detect agglutination sufficiently accurately to avoid manual verification. Indeed, existing automated cell counters erroneously measure agglutinated clumps of cells as one large cell producing an inaccurate mean cell volume and cell count and compound indices derived from them. An abnormally high mean corpuscular volume (MCV) or an abnormally elevated mean corpuscular haemoglobin concentration (MCHC) displayed by commercial haematology autoanalysers alerts the technician to the

possibility of the presence of agglutination. However, these indices are inadequate indicators of agglutination because they are not specific, moreover agglutination must rise to high levels before the indices exceed the normal limits. An elevated MCHC is produced by red cell fragmentation, lymphocytosis, hyperglycemia and haemoglobinaemia and therefore requires manual inspection and further testing to establish the diagnosis.

In conventional laboratories which perform blood typing and cross-matching, to determine the blood group of a sample one or two drops of existing commercially available blood group antibodies are added to neat whole blood, or more usually a 3 to 5% suspension of whole blood in normal saline. The suspension is incubated at room temperature for some minutes, typically 2 or 3 minutes. The suspension is then centrifuged for 30 to 45 seconds at 3000 rpm in a bench-top centrifuge. The suspension is then gently shaken for a few seconds. The tube is then examined visually for the presence of agglutinated cells and confirmed using low-powered microscopy. Cross-matching is performed in the same way using the recipient's plasma as the antibody in place of commercial antibody to confirm that there is no agglutination.

Summary of the Invention

According to the present invention, a method of detecting agglutination in a sample of cells comprises the steps of inducing cells to change at least one of their properties so as to separate agglutinated cells and detecting the resultant alteration in the cell population.

Preferably, the property change is that of the shape of the cells. More preferably, the cell sample is subject to an alteration in environment to cause the cells to sphere. In a preferred example, the alteration in the environment is a change in osmolality of a liquid medium in which the cells are suspended, preferably by the addition of water.

Preferably, alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor. More preferably, the sample is fed continuously into a solution the osmolality of which is changed continuously to produce a continuous series of aliquots of cells which are passed through the sensor.

Preferably, the method further comprises the step of pre-treating the sample of cells to induce, or at least attempt to induce, agglutination. In one preferred use of the invention, a cell sample of unknown antigenicity from one source is mixed with antibodies from a different source. The antibodies may be manufactured or come from whole blood, plasma or typically serum. In a further step, the putative antigen-antibody mixture may be tested at different temperatures to reveal heat sensitive agglutination. Accordingly, a new test is provided which can replace existing blood grouping and cross matching techniques.

The present invention measures agglutination using a process which is also capable of testing how tightly agglutinated cells are bonded by measuring how much force is required to separate them. This property depends upon antibodies interacting with the complement system. Agglutination of red blood cells is a function of the type and

number of antigen combining sites on the surface of the cells, which bind with complementary Gig antibody molecules. The strength of agglutination is a function of the proximity of the binding sites on the cell surface. By placing a whole blood sample into a typically 1:10,000 suspension, and causing cells which are approximately bi-concave discs to sphere, the effective surface area available for bonding diminishes. Sphering a cell increases the space between antigen binding sites and increases the mean distance across which bonding occurs. The surface area available for bonding between cells decreases as cells sphere hence they lose bonding strength and separate. By recording the inducing pressure and the number of cells (or quantities related to it) as they change with respect to the inducing pressure, agglutination can be detected, quantified and monitored. Cells which have agglutinated, when tested by this method, separate and thereby increase the cell count in a characteristic fashion. In a further step the sample is subject to mechanical agitation which tends to promote agglutination in normally shaped cells capable of agglutination but promotes separation of spherically shaped cells.

Brief Description of the Drawings

Examples of the present invention will now be described in detail with reference to the accompanying drawings, in which:

Figure 1 is a screen dump of a set of results from an automatic blood cell analyzer of the type described in detail in International patent application WO97/24601, for a patient having normal non-agglutinated blood cells;

Figure 2 is a similar screen dump of a set of results for a patient having agglutinated blood cells; and,

Figure 3 is another screen dump showing the results of mixing a sample of blood with antibodies in a test to determine blood type.

Detailed Description

The method of the present invention is exceptionally useful in conjunction with the methods and apparatus described in the applicants' earlier filed International patent applications, namely WO97/24601, WO97/24598 and WO97/24599, and enhances the general utility of the tests described therein.

The preferred method consists of counting the cells as they pass through an aperture. The instrument may be configured with a mixing chamber into which saline, cells and diluent are injected, in which case the number of cells passing through the aperture at every osmolality does not vary. When only two streams are injected into the mixing chamber, diluent and a saline suspension into which the cells have been previously introduced, the number of cells passing through the aperture is fixed at a level that is directly proportional to the osmotic gradient. Since the red blood cells suspended in a liquid medium are exposed to a progressive reduction in ambient osmolality, and the method normally injects a progressively smaller stream of cells into the mixing chamber, a progressive reduction in cell count is observed.

The results generated by the instrument described in International Patent Application WO 97/24601 for a normal patient are shown in Figure 1. In Figure 1, as well as in Figures 2 and 3, in area A the plot represents the red cell count. Deviation from the predetermined straight line of cell count against osmolality (as shown in area A of

Figure 2) can only occur if additional particles appear, or are stimulated by the ambient change in pressure.

As will be described below, when cells agglutinate or are made to agglutinate, the cell count falls; then, as the cells sphere, the cell count increases with each aggregate tending to separate into its component parts in inverse proportion to the strength of the agglutination.

Most cells sphere in the range of pressures in the interval between P_{max} and P_0, where P_{max} is the point at which the rate of fluid flow into the cell reaches a maximum and P_0 is the equilibrium point (see area B). If agglutinated clumps are present they will separate in the same interval causing a local increase in count. Furthermore, the point at which P_0 occurs gives an indication of whether or not agglutination is occurring, since the point at which P_0 occurs increases if cells are agglutinating.

Our corresponding International application (Agent's reference G14201WO) discloses a method of measuring cell fragments. Fragments and disrupted agglutinated cells (DACs) can be segregated by size. Fragments are quite small between 10 and 30 fl in volume whereas DACs are at least three times the size, generally between 60-110 fl. In addition, the isotonic MCV is normal or reduced in the presence of fragments while the MCV is elevated with agglutination. As the normal range of MCV is so large it can hide much agglutination.

Sample ageing and the application of mechanical, ultrasound or other stress increases

the count of intact cells if the sample was agglutinating and decreases the number of intact cells if the sample is fragmenting. Dropping the ambient osmolality below P_0 has no further disrupting effect on agglutinated clumps but the frequency of cell fragments have been found to vary inversely with osmolality.

Figure 2 shows the results for a patient having agglutinated blood cells. The sudden increase in cell count at sphering is shown clearly in area A, and the increased sphericity index (SI) appears as a fat cell in area B. SI can also be seen from the Table (area C). A sphere has a SI of 10 whereas a flatter cell has a higher SI. In Figure 2 (abnormal patient) the value of SI is 10.24 whereas in Figure 1 (healthy patient) the corresponding value is 14.37.

Area D in Figure 2, in comparison with Figure 1, shows the increase in variance of the red cell frequency distribution due to agglutinated clumps of cells. An analysis of the frequency distribution provides an indication of whether or not the cells are agglutinating. Firstly, the width of the distribution, as measured by the standard deviation (SD), or coefficient of variation (cv), increases with agglutination. Secondly, any deviation from a normal distribution can be measured. A bias away from the centre leading to a flatter shaped curve, termed negative kurtosis, provides an indication of agglutination. Comparing area D in Figures 1 and 2 shows that in the abnormal patient the standard deviations are about twice the normal and kurtosis is negative.

Area E shows frequency distributions indicating the profile of cell size measurement

against increasing osmolality. As the stress is increased the cells begin to swell resulting in the gradual increase in mean cell size. At P_0 the cell size can increase no more, and upon a further increase in stress, the cells evacuate their contents to become "ghost cells".

As an example, the method may be embodied into an instrument for the automated recognition of blood groups and cell types by the induction and detection of agglutination by introducing antibodies (for example, using any one or more of the commercially available antibodies currently used for blood typing purposes, or using the recipient's plasma as the antibody source for the purpose of cross-matching) into the water syringe which subsequently meets the saline blood suspension in the mixing chamber. Agglutination caused by the interaction of the antibodies and the antigens on the surface of the cells, or the lack of it, is detected at the sensor aperture by counting. This test eliminates the need for manual blood grouping and cross-matching.

The results generated by such an instrument described in International patent application WO97/24601 are shown in Figure 3. In this example, the fluids were warmed within the apparatus to a temperature of around 37°C ie body temperature, to stimulate normal body environment. Agglutination is recognisable by the presence of an increase in the red cell count, usually between P_{max} and P_{min}, by the frequency distribution (in this example the isotonic, spherical, ghost, and "user" selected frequency distributions are taken at respective sampling instants shown by the "H"s in area E) showing negative kurtosis, by an increase in the distribution width of the

cell population measured by an increase in the standard deviation or coefficient of variation, by an increase in the sphericity index, and by an increase in the osmolality which induces zero permeability (P_0). Any change, even minor change (detectable when compared with a control run without antibodies) must be attributable solely to the antibody. In this particular example, a second population of cells is visible on both areas B and E in Figure 3, which also indicates agglutination.

The present invention is particularly useful in the early detection of agglutination, hence the early detection and subsequent treatment of haemolytic diseases, and enhanced possibility of recognizing the underlying pathology. It is also possible to quantify the strength of cell agglutination from the extent to which separation is achieved and the ease with which it is achieved. As the unagglutinated cell concentration is known any reduction in the isotonic count represents agglutination. As the cell suspension is exposed to the sphering gradient, the original count will be restored at higher osmolalities and in proportion to the strength of the agglutination. Finally, the method provides for the automatic identification of blood groups and cell types by inducing cells to agglutinate and subsequently testing them using the method.

CLAIMS

1. A method of detecting agglutination in a sample of cells, comprising the steps of inducing the cells to change at least one of their properties so as to separate agglutinated cells and detecting the resultant alteration in the cell population.

2. A method according to claim 1, comprising the step of measuring the force required to separate agglutinated cells.

3. A method according to claim 1 or 2, in which the property changed is that of the shape of the cells.

4. A method according to any preceding claim, in which the cell sample is subject to an alteration to cause the cells to sphere.

5. A method according to claim 4, in which the alteration is a change in osmolality of a liquid medium in which the cells are suspended.

6. A method according to any preceding claim, in which alterations in the cell population are detected by passing one or more aliquots of the cell sample through a sensor which is adapted to count the number of cells passing through the sensor.

7. A method according to claim 6, in which the sample is fed continuously into a solution the osmolality of which is changed continuously to produce a continuous

series of aliquots of cells which are passed through the sensor.

8. A method according to any preceding claim, further comprising the step of pretreating the sample of cells to induce, or at least attempt to induce, agglutination.

9. A method according to any preceding claim, in which the cell sample is obtained from a source of whole blood.

10. A method according to claim 9, in which the sample of cells are treated with antibodies from a different source.

11. A method according to claim 10, in which the cells are treated in order to determine the blood type.

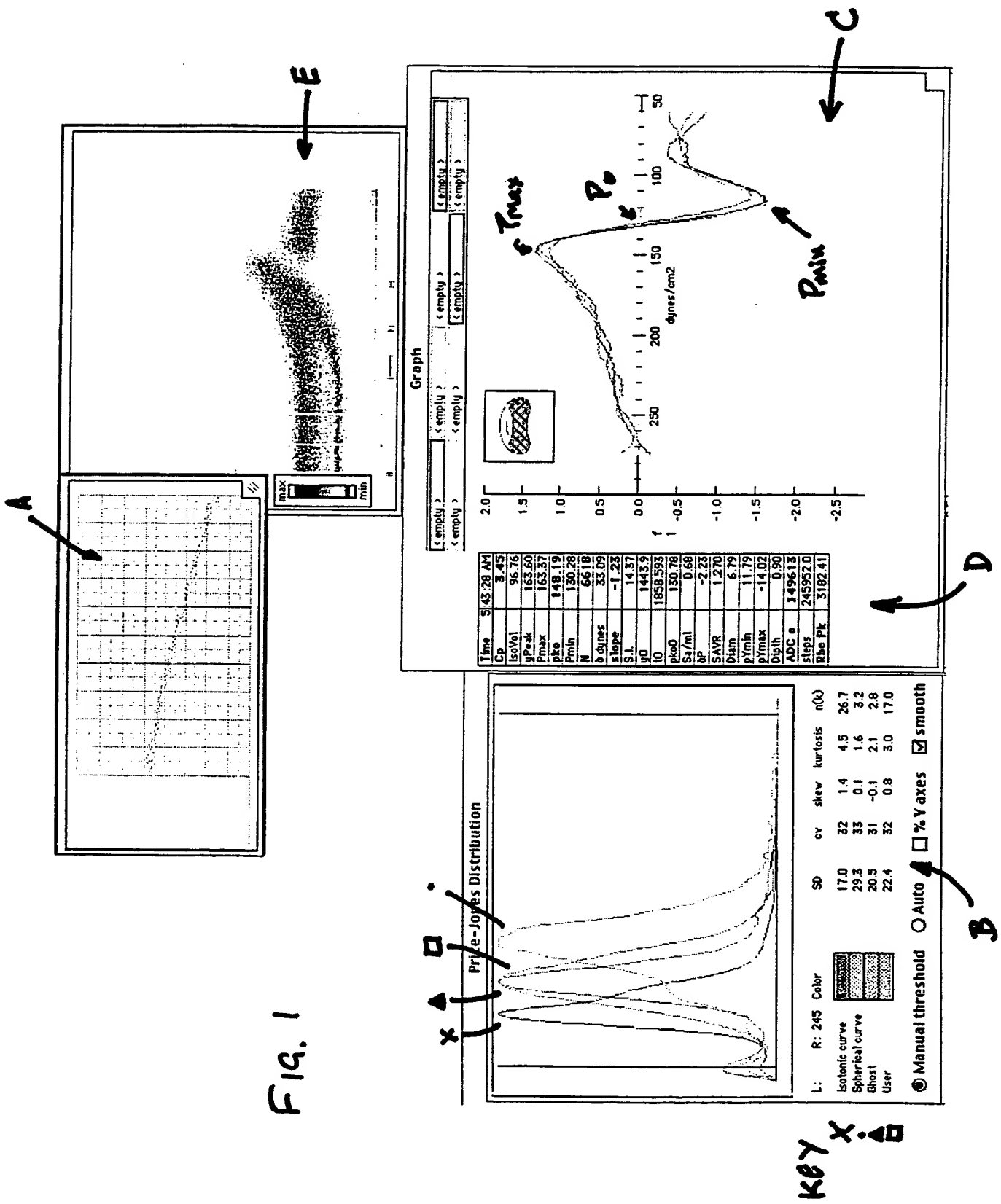
12. A method according to claim 10, in which the cells are treated in order to cross-match the sample.

13. A method according to any of claims 10 to 12, in which the antibodies from the different source are manufactured, or come from whole blood, plasma or serum.

14. A method according to any of claims 8 to 13, in which the sample of cells is pre-treated by exposure to heat.

15. A method according to any of claims 8 to 14, in which the sample is warmed to a temperature of between 35°C to 40°C, preferably 37°C.

16. A method according to any of claims 8 to 13, in which the sample of cells is pre-treated by cooling the sample.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Rbe Pk 3182.41
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Spherical curve 29.3 33 0.1 1.6 3.2
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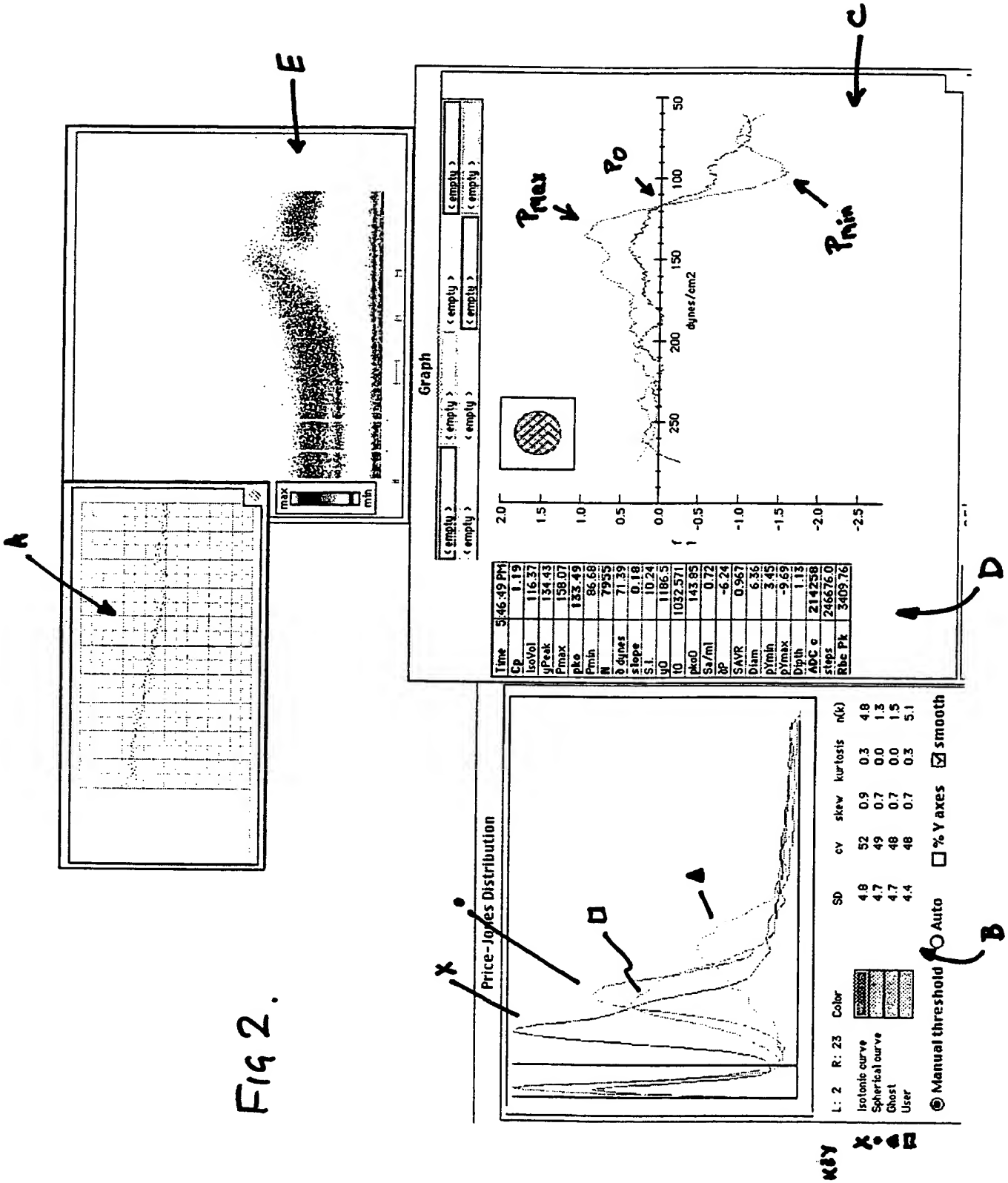
KEY

FIG. 1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Pmax 158.07
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t0 1032.571
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Spherical curve 4.7 49 0.7 0.0 1.3
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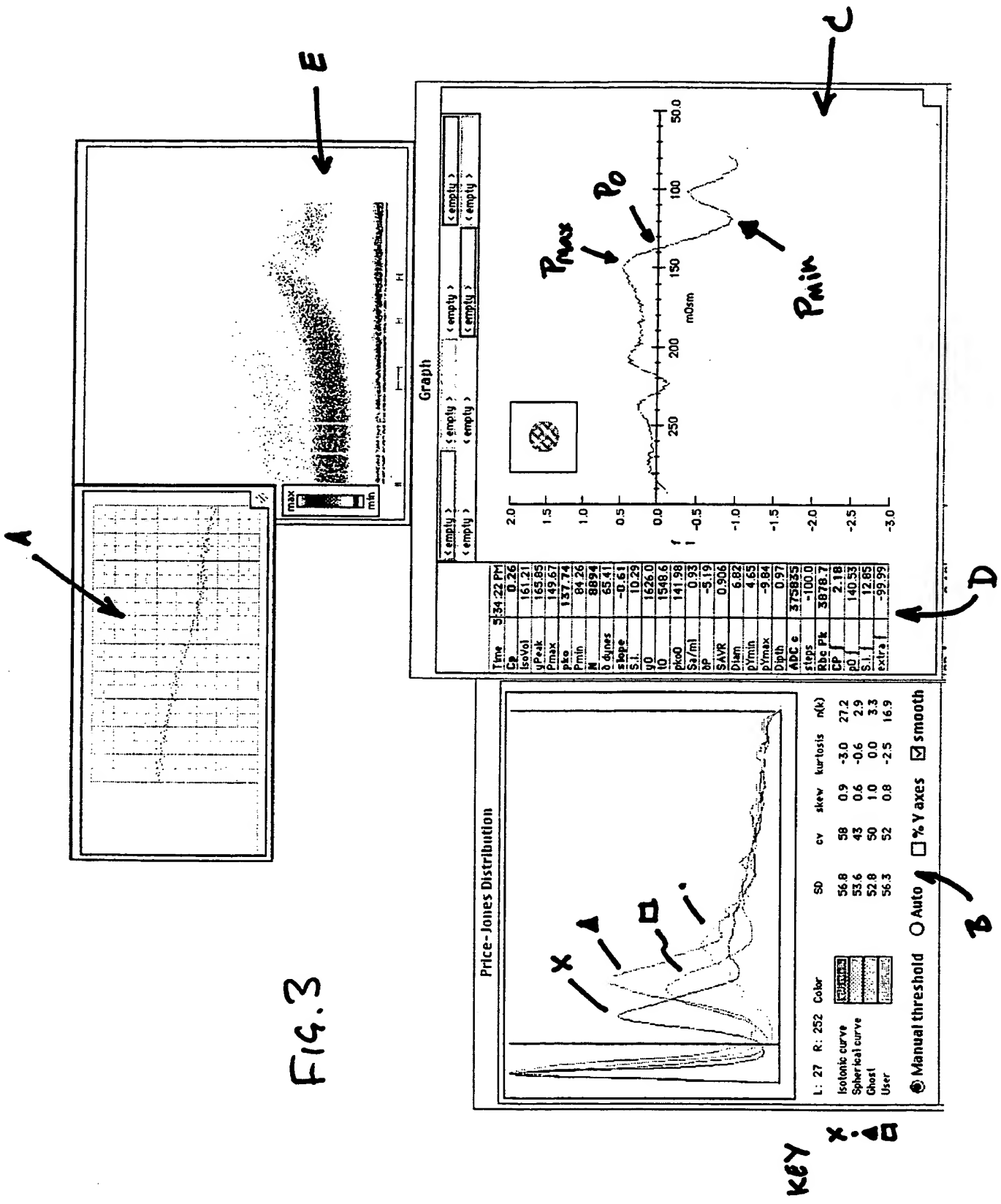
Auto
% Y axes
smooth
key

Fig 2.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Pmin
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KEY
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% Y axes
smooth
Isotonic curve
Spherical curve
Ghost
User

FIG.3

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IMPORTANT NOTIFICATION

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB 99/04438	24/12/1999	29/12/1998

Applicant: SHINE, IAN BASIL et al.

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2. ☐ The search copy was accompanied by a nucleotide and/or amino acid sequence listing in computer readable form.

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(PCT Article 18 and Rules 43 and 44)

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International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/GB 99/ 04438	24/12/1999	29/12/1998
Applicant		
SHINE, IAN BASIL et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

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   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

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   [ ] filed together with the international application in computer readable form.

   [ ] furnished subsequently to this Authority in written form.

   [ ] furnished subsequently to this Authority in computer readble form.

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   [ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   [X] the text is approved as submitted by the applicant.

   [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   [ ] as suggested by the applicant.

   [ ] because the applicant failed to suggest a figure.

   [ ] because this figure better characterizes the invention.

   [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted] 99/04438

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N15/02 G01N33/80 G01N33/49 G01N33/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 4 900 685 A (SMITH III NATHAN L) 13 February 1990 (1990-02-13) column 5, line 63 -column 6, line 15 column 14, line 27-30 ---	1
A	US 5 510 261 A (GOOCHEE CHARLES F ET AL) 23 April 1996 (1996-04-23) column 9, line 21-24 ---	1
A	US 5 064 765 A (KARASIKOV NIR ET AL) 12 November 1991 (1991-11-12) abstract ---	1
A	US 5 350 652 A (ANTONIADES MICHAEL G) 27 September 1994 (1994-09-27) column 4, line 3 -column 5, line 48 --- -/--	1

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 June 2000	04/07/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Zinngrebe, U

1

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PC[redacted]B 99/04438

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	GB 2 186 684 A (LONDON POLYTECH) 19 August 1987 (1987-08-19) figure 3A -----	1

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 99/04438

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
US 4900685	A	13-02-1990	AT	107418 T	15-07-1994
			AU	610564 B	23-05-1991
			AU	1245188 A	24-08-1988
			CA	1305409 A	21-07-1992
			DE	3850249 D	21-07-1994
			EP	0345277 A	13-12-1989
			JP	2501948 T	28-06-1990
			WO	8805913 A	11-08-1988
			US	5494800 A	27-02-1996
US 5510261	A	23-04-1996	AU	3144193 A	15-06-1993
			WO	9310260 A	27-05-1993
US 5064765	A	12-11-1991	DE	4006595 A	06-09-1990
			GB	2230604 A	24-10-1990
			JP	2284061 A	21-11-1990
US 5350652	A	27-09-1994	DE	69421659 D	23-12-1999
			DE	69421659 T	08-06-2000
			EP	0645669 A	29-03-1995
			JP	7172830 A	11-07-1995
GB 2186684	A	19-08-1987	GB	2191280 A	09-12-1987

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

NICHOLLS, Michael, John
J.A. Kemp & Co.
14 South Square
Gray's Inn
London WC1R 5LX
ROYAUME-UNI

Date of mailing (day/month/year)
13 November 2000 (13.11.00)

Applicant's or agent's file reference
N.75739A MN

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/04338

International filing date (day/month/year)
21 December 1999 (21.12.99)

1. The following indications appeared on record concerning:

[X] the applicant [ ] the inventor [ ] the agent [ ] the common representative

Name and Address
DASH TECHNOLOGIES LIMITED
52 New Inn Hall Street
Oxford OX1 2QA
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [ ] the name [ ] the address [ ] the nationality [ ] the residence

Name and Address
CELOXICA LIMITED
7 Milton Park
Abingdon
Oxfordshire OX14 4RT
United Kingdom

State of Nationality: GB
State of Residence: GB
Telephone No.:
Facsimile No.:
Teleprinter No.:

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
R. Chrem
Telephone No.: (41-22) 338.83.38

F ENT COOPERATION TRE/

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and Administrative Instructions, Section 422)

To:

FINNIE, Peter, John
Gill Jennings & Every
Broadgate House
7 Eldon Street
London EC2M 7HL
ROYAUME-UNI

Date of mailing (day/month/year)
13 November 2000 (13.11.00)

Applicant's or agent's file reference
PJF/G14202WO

IMPORTANT NOTIFICATION

International application No.
PCT/GB99/04438

International filing date (day/month/year)
24 December 1999 (24.12.99)

1. The following indications appeared on record concerning:

[ ] the applicant [ ] the inventor [X] the agent [ ] the common representative

Name and Address
Elkington and Fife
Prospect House
8 Pembroke Road, Sevenoaks
Kent TN13 1XR
United Kingdom

State of Nationality | State of Residence

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

[X] the person [X] the name [X] the address [ ] the nationality [ ] the residence

Name and Address
FINNIE, Peter, John
Gill Jennings & Every
Broadgate House
7 Eldon Street
London EC2M 7HL
United Kingdom

State of Nationality | State of Residence

Telephone No.
020 7377 1377

Facsimile No.
020 7377 6036

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

[X] the receiving Office
[ ] the International Searching Authority
[X] the International Preliminary Examining Authority
[ ] the designated Offices concerned
[X] the elected Offices concerned
[ ] other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland
Facsimile No.: (41-22) 740.14.35

Authorized officer
Maria Victoria CORTIELLO
Telephone No.: (41-22) 338.83.38

WO 00/39560
PCT/GB99/04438

PCT NT COOPERATION TREA

From the INTERNATIONAL BUREAU

PCT

NOTICE INFORMING THE APPLICANT OF THE COMMUNICATION OF THE INTERNATIONAL APPLICATION TO THE DESIGNATED OFFICES

(PCT Rule 47.1(c), first sentence)

To:
ELKINGTON AND FIFE
Prospect House
8 Pembroke Road
Sevenoaks
Kent TN13 1XR
ROYAUME-UNI

Date of mailing (day/month/year) 06 July 2000 (06.07.00)	
Applicant's or agent's file reference PJF/G14202WO	**IMPORTANT NOTICE**

International application No.	**International filing date** (day/month/year)	**Priority date** (day/month/year)
PCT/GB99/04438	24 December 1999 (24.12.99)	29 December 1998 (29.12.98)

Applicant
SHINE, Ian, Basil et al

1. Notice is hereby given that the International Bureau has communicated, as provided in Article 20, the international application to the following designated Offices on the date indicated above as the date of mailing of this Notice:
   AU,CN,JP,KP,KR,US

   In accordance with Rule 47.1(c), third sentence, those Offices will accept the present Notice as conclusive evidence that the communication of the international application has duly taken place on the date of mailing indicated above and no copy of the international application is required to be furnished by the applicant to the designated Office(s).

2. The following designated Offices have waived the requirement for such a communication at this time:
   AE,AL,AM,AP,AT,AZ,BA,BB,BG,BR,BY,CA,CH,CR,CU,CZ,DE,DK,DM,EA,EE,EP,ES,FI,GB,GD,GE,
   GH,GM,HR,HU,ID,IL,IN,IS,KE,KG,KZ,LC,LK,LR,LS,LT,LU,LV,MA,MD,MG,MK,MN,MW,MX,NO,NZ,
   OA,PL,PT,RO,RU,SD,SE,SG,SI,SK,SL,TJ,TM,TR,TT,TZ,UA,UG,UZ,VN,YU,ZA,ZW
   The communication will be made to those Offices only upon their request. Furthermore, those Offices do not require the applicant to furnish a copy of the international application (Rule 49.1(a-bis)).

3. Enclosed with this Notice is a copy of the international application as published by the International Bureau on
   06 July 2000 (06.07.00) under No. WO 00/39560

REMINDER REGARDING CHAPTER II (Article 31(2)(a) and Rule 54.2)

If the applicant wishes to postpone entry into the national phase until 30 months (or later in some Offices) from the priority date, **a demand for international preliminary examination** must be filed with the competent International Preliminary Examining Authority before the expiration of 19 months from the priority date.

It is the applicant's sole responsibility to monitor the 19-month time limit.

Note that only an applicant who is a national or resident of a PCT Contracting State which is bound by Chapter II has the right to file a demand for international preliminary examination.

REMINDER REGARDING ENTRY INTO THE NATIONAL PHASE (Article 22 or 39(1))

If the applicant wishes to proceed with the international application in the **national phase**, he must, within 20 months or 30 months, or later in some Offices, perform the acts referred to therein before each designated or elected Office.

For further important information on the time limits and acts to be performed for entering the national phase, see the Annex to Form PCT/IB/301 (Notification of Receipt of Record Copy) and Volume II of the PCT Applicant's Guide.

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer J. Zahra
Facsimile No. (41-22) 740.14.35	Telephone No. (41-22) 338.83.38

PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 August 2000 (25.08.00)	
International application No. PCT/GB99/04438	**Applicant's or agent's file reference** PJF/G14202WO
International filing date (day/month/year) 24 December 1999 (24.12.99)	**Priority date** (day/month/year) 29 December 1998 (29.12.98)
Applicant SHINE, Ian, Basil et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

28 July 2000 (28.07.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Juan Cruz
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

REC'D 17 NOV 2000
WIPO PCT

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
PJF/G14202WO		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04438	24/12/1999	29/12/1998
International Patent Classification (IPC) or national classification and IPC G01N15/02		
Applicant SHINE, IAN BASIL et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
28/07/2000	15.11.2000
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Tilkorn, A-C Telephone No. +49 89 2399 8688

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/04438

I. Basis of th report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
Description, pages:

1-9 as originally filed

Claims, No.:

1-16 as originally filed

Drawings, sheets:

1/3-3/3 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in th language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in th cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:

☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-16
	No:	Claims	-
Inventive step (IS)	Yes:	Claims	1-16
	No:	Claims	-
Industrial applicability (IA)	Yes:	Claims	1-16
	No:	Claims	-

2. Citations and explanations
see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
s e separate sheet

Re Item V

The following documents are referred to in this communication:

D1: US-A-4 436 827
D2: WO 97 24601 A

D1 is cited by the Examiner (Guidelines VI 7.24). D2 is cited in the application (appl.: e.g. p 4 l 21, p 5 l 7). A copy of each of D1 and D2 is appended to this report.

1 Novelty (Art 33(2) PCT):

Claim 1 is novel, because none of the available documents describes a method of detecting agglutination by separating the agglutinated cells and detecting the resultant alteration in the cell population. Dependent **claims 2-16** are novel, accordingly.

2 Inventive Step (Art 33(3) PCT):

Claim 1 appears to satisfy Art 33(3) PCT for the following reasons:
D1 which is considered to represent the closest prior art describes a method of detecting agglutination on the basis of separating agglutinated cell complexes from non-agglutinated suspension by particle size (D1: col 2 l 18-31), but D1 does not disclose a method which leads to the separation of the agglutinated cells.
The problem to be solved can thus be regarded as the provision of an alternative method of detecting agglutination.
Although D2 discloses a method comprising inducing the cells to change one of their properties, namely the shape and detecting the resultant alteration in the cell population (D2: p 3 l 20-35), there is no indicator found throughout either of D1 or D2 which points towards the detection of agglutination using the method steps disclosed in D2. More specifically, neither D1 nor D2 describe the separation of agglutinated cells by altering the shape of the cells.
Thus, **claim 1** seems to satisfy Art 33(3) PCT. The same applies to dependent **claims 2-16**, accordingly.

Re Item VII

Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the document D1 not mentioned in the description, nor is this document identified therein.

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

ELKINGTON & FIFE
Prospect House
8 Pembroke Road
Sevenoaks
Kent TN13 1XR
GRANDE BRETAGNE

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

	Date of mailing *(day/month/year)* 15.11.2000
Applicant's or agent's file reference PJF/G14202WO	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/GB99/04438	24/12/1999	29/12/1998

Applicant
SHINE, IAN BASIL et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Danti, B Tel.+49 89 2399-8161

Form PCT/IPEA/416 (July 1992)